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(71) Applicant
Leonard Maitland McEwan
Weir View, Wargrave Road, Henley-on-Thames, Oxfordshire

(72) Inventor
Leonard Maitland McEwan

(74) Agent and/or Address for Service
Stevens Hewlett & Perkins
5 Quality Court, Chancery Lane, London, WC2A 1HZ

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(54) Beta-glucuronidase containing compositions

(57) Compositions containing the enzyme β–glucuronidase are useful for the treatment of certain chronic viral infections, such as Acquired Immunodeficiency Syndrome and Myalgic Encephalopathy. Preferably, the compositions comprise a sterile aqueous solution of β–glucuronidase, 1,3–cyclohexane diol and protamine.

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Beta-Glucuronidase Containing Compositions

The present invention relates to beta-glucuronidase containing compositions. More particularly, it relates to beta-glucuronidase compositions for use in the treatment of certain chronic virus infections such as Acquired Immunodeficiency Syndrome and those commonly referred to collectively as the post-viral syndrome.

The post-viral syndrome (otherwise known as Myalgic Encephalopathy) is thought to be principally caused by chronic infection with Coxsackie virus (classically Coxsackie B4), or with Epstein Barr virus. Other cases appear to be caused by chronic infection with unidentified viruses. Patients with post-viral syndrome often exhibit symptoms of food intolerance. Patients infected with Human Immunodeficiency Virus (HIV), in the later stages of the carrier stage, suffer chronic ill-health, often involving intestinal and skin complications.

The present invention is based on the discovery that the treatment of patients suffering from such chronic viral infections using certain compositions containing beta-glucuronidase can bring about an amelioration of the symptoms of such infections.

Beta-glucuronidase is known to have the ability to potentiate the desensitising effect of low doses of allergens, such as pollen extract, in humans and can be used for the prophylactic treatment of hay fever in humans, see L.M. McEwen et al, "Ann. Allerg.", 31, (1973), p 543-550. The authors, in that reference, showed that the immunological effect of beta-glucuronidase is controlled by the presence of any substance which contains at least two hydroxyl groups, particularly 1,3-diols. L. M. McEwen et al, in "Ann. Allerg.", 34, (1975), p 290-295, showed that the

immunological activity of beta-glucuronidase is also affected by the presence of protamine such that beta-glucuronidase/1,3-cyclohexane diol compositions containing protamine require lower concentrations of the diol, compared to protamine-free compositions, to achieve the same immunological effect. The treatment of patients with acute food allergy by enzyme potentiated hyposensitisation using formulations containing beta-glucuronidase, 1,3-cyclohexane diol and protamine is described by L.M. McEwen in Ann. Allerg., 35, (1975) p 98-103.

According to a first aspect, the present invention provides the use of beta-glucuronidase for the manufacture of a composition for use in the treatment of post viral syndrome which composition comprises a sterile aqueous solution of highly purified beta-glucuronidase.

According to a second aspect, the invention provides the use of beta-glucuronidase for the manufacture of a composition for use in the treatment of Acquired Immunodeficiency Syndrome which composition comprises a sterile aqueous solution of highly purified beta-glucuronidase.

Typically, according to the aspects described above, the concentration of beta-glucuronidase in the treatment solution, will be in the range of from 50 to 1000 Fishman units, preferably about 400 Fishman units, per dose delivered to the patient.

In a preferred embodiment, the compositions useful for treating patients suffering from post viral syndrome or HIV infections will additionally contain a compound containing at least two hydroxyl groups for controlling the immunological activity of the beta-glucuronidase. Such compounds which, of course, will be non-toxic to the human body in the concentrations used will typically be those having at least two hydroxyl

groups situated on adjacent carbon atoms or separated by upto four carbon atoms. Such diverse substances as mucopolysaccharides, glucose and propylene glycol have the ability to control the immunological activity of beta-glucuronidase. However, 1,3 diols are preferred since they are capable of affecting the activity of the enzyme at much lower concentrations. Of the 1,3-diols useful in the present invention, good results may be obtained using 1,3-cyclohexane diol in a concentration in the range of from 3×10^{-10} to 1×10^{-11} g per dose delivered to the patient. Thus, in a typical dose of 0.1 ml injected intradermally into a patient the concentration above corresponds to an actual concentration of from 3×10^{-9} to 1×10^{-10} g/ml.

The immunological and enzymatic activity of purified beta-glucuronidase may also be controlled by varying concentrations of certain diamines (e.g. 1,10-diaminodecane) and polyamines. In the present invention, however, it is preferred to exploit the activity of hydroxyl-containing compounds as described above while ensuring a large and predictable polyamino effect by the addition of protamine. For compositions containing protamine as an activator in addition to the 1,3-diol potentiator and the beta-glucuronidase the optimal dose of the diol is lower than in the case where no protamine is used. In the compositions described above, protamine is preferably added at a typical concentration in the range of from 1×10^{-8} to 1000×10^{-8} g per dose delivered to the patient.

It is highly preferred, in the present invention, to treat patients with post-viral syndrome or HIV infection with features suggesting hypersensitivity using formulations containing beta-glucuronidase, 1,3-cyclohexane diol and protamine. Thus, a particular preferred embodiment of the present

invention provides the use of a composition comprising beta-glucuronidase, 1,3-cyclohexane diol and protamine for the manufacture of a preparation having use in the treatment of post-viral syndrome or acquired immunodeficiency syndrome wherein the preparation comprises a sterile aqueous phosphate-free buffer solution containing from 50 to 1000 Fishman units of beta-glucuronidase, from 1×10^{-10} to 3×10^{-9}g of 1,3-cyclohexane diol and from 1×10^{-8} to 1×10^{-5} g protamine, all concentrations being per dose to be delivered to the patient.

In the above preparation, the buffer used is a phosphate-free buffer, preferably a sodium acetate/HCl buffer having pH about 6.9 to ensure optimum stability of the ingredients of the preparation. The preparation above may be administered to patients with or without allergens or allergen extracts. Allergen extracts such as are used in the known enzyme potentiated desensitisation treatment of allergies may also be incorporated into the treatment preparations described above, if desired, in order to bring about desensitisation in the patient. Allergen extracts when used will be present in extremely low concentrations, varying from 1×10^{-6} g per dose down to about 1×10^{-14} g per dose depending on which allergen is employed and which allergic syndrome is being treated. For purified allergens of known molecular weight (e.g. food additives) doses containing as low as about 100 molecules can be used to good effect. The production and storage of such high dilutions requires the use of carrier molecules, and, for this purpose, chondroitin-6-sulphate is very suitable. However, other substances, such as heparin, may be used instead as the allergen carrier. Chondroitin sulphate incorporated into a formulation administered to a patient has the effect of altering the activation of that formulation and,

therefore, affects the optimum doses of protamine and diol. Typically chondroitin sulphate, if used, will be present in an amount of from 1 to 0.01 mg per dose.

It has been found that formulations as described above, when administered to patients with post-viral syndrome exhibiting symptoms of food intolerance, produce a major overall improvement in symptoms which persists for several weeks. When the effect disappears the patient cannot be restored to the same degree of well-being by avoidance of the foods to which he/she is intolerant. Thus, it is believed that the beta-glucuronidase formulation confers an extra major benefit which is independent of the food allergens with which it was administered. In this role, the beta-glucuronidase formulation is directly ameliorating the symptoms of post-viral syndrome but it is not stimulating the patient's immune system to rid him/her of the persistent viral infection. It appears that tolerance is induced to some facets of the effect of the chronic virus infection.

Although the most important effect of this tolerising treatment is independent of antigens added to the formulation (the virus antigen present within the patient is sufficient), patients suitable for this treatment will often require enzyme potentiated desensitisation to food antigens at the same time.

In addition to the above, it has been discovered that if cyclohexane diol is present in each dose of the beta-glucuronidase formulation delivered to the patient at a concentration of from 1×10^{-8} to 1×10^{-7}g, and this formulation is administered with a suitable dose of antigen, the effect is hypersensitisation; the reverse of immune tolerance. This discovery forms the basis for a separate aspect of the present invention which provides the use of a composition comprising beta-glucuronidase, 1,3-

cyclohexane diol and protamine for the manufacture of a formulation for increasing the cellular immune response in humans to immunising vaccines wherein the formulation comprises a sterile aqueous phosphate-free buffer solution containing from 50 to 1000 Fishman units of beta-glucuronidase, from 1×10^{-8} to 1×10^{-7} g of 1, 3-cyclohexane diol, from 1×10^{-8} to 1×10^{-5} g of protamine and a suitable dose of antigen, all concentrations being per dose delivered to the patient. It has further been discovered that if the above enzyme/diol/protamine formulation (without antigen) is administered to patients suffering from long standing chronic virus infections,the treatment stimulates the patients immunity causing resolution of the chronic infection. This effect is observed in patients suffering from post-viral syndrome and in other chronic virus infections, such as virus induced chronic prostatitis and recurrent herpes.

Additionally, it has now been discovered that patients infected with HIV may also be treated in the ways described above. The later stages of the carrier state can be improved much like Coxsackie B4 post-viral syndrome. For this purpose, a desensitising formulation would be used containing beta-glucuronidase and 1,3-cyclohexane diol with or without allergens. When appropriate, this treatment may be coupled to nutritional therapy, anti-candida therapy and allergen avoidance. Alternatively, any phase of HIV infection may be improved by the administration to the patient of a hypersensitising formulation of beta-glucuronidase and 1,3-cyclohexane diol.

EXAMPLE 1

Formulations suitable for treatment of patient with post-viral syndrome or HIV infection with features suggesting hypersensitivity

Beta-glucuronidase, 400 Fishman units
1,3 cyclohexane diol, 3×10^{-10} g
Protamine 2×10^{-6} g
Antigens 1×10^{-10} to 1×10^{-14} g) (Optional)
Chondroitin sulphate 0.1 mg)

Injected intradermally in 0.2 ml of sodium acetate/HCl buffer solution, pH 6.9.

EXAMPLE 2

Formulations suitable for increasing the cellular immune response to immunising vaccines

Beta-glucuronidase,400 Fishman units
1, 3 cyclohexane diol, 3×10^{-8} g
Protamine 2×10^{-6} g
Suitable dose of antigen

Inject intradermally in 0.2 ml of sodium acetate/HCl buffer solution, pH 6.9.

EXAMPLE 3

Formulations suitable for treatment of viral carrier state

Beta-glucuronidase, 400 Fishman units
1,3 cyclohexane diol, 3×10^{-8} g
Protamine 2×10^{-6} g
Inject intradermally in 0.1 ml of sodium acetate/HCl buffer solution, pH 6.9.

EXAMPLE 4

Treatment of patient infected by Human Immunodeficiency Virus

The patient is male, aged 32 years. Probable date of infection 4 years previously. Non-specific ill-health for past 10 months. Diagnosis made one year before the treatment with beta glucuronidase. Given 3 treatments according to formulation of example 3 by intradermal injections.

The lymphocyte counts and treatment dates were as follows:

(Laboratory normal ranges		1500-4000	815-2480	280-1350)
Date	Treatment	Total Lymphocytes	T4 (Helper)	T8 (Suppressor)
23 Jul	-	3306	661	2248
4 Aug	Dose 1			
25 Aug	-	3876	271	3458
17 Sept	-	2989	209	2152
23 Sept	Dose 2			
27 Oct	Dose 3			
3 Dec		5049	1009	3839

Interpretation:

The patient already had raised numbers of T8 lymphycytes. The first dose of activated beta glucuronidase has stimulated the T8 cells, especially those with killer function. Cells of the T4 subset which were already virus-infected (and therefore functionless) have been eliminated.

Further treatment stimulates replication of both T4 and T8 subsets with recovery of T4 numbers.

In September the patient, who had regained his well-being, suffered an attack of influenza from which he recovered quickly without treatment or complication. It is possible that the T8 cells had been influenced by the beta glucuronidase to adopt some helper functions.

The raised T8 lymphocyte count before treatment is typical of concurrent cytomegalovirus (CMV) infection. At the conclusion of treatment the CMV -IgM test was negative. At that stage the elevated T8 lymphocyte counts appeared to be responses to the beta glucuronidase treatments alone.

CLAIMS

1. The use of beta-glucuronidase for the manufacture of a composition for use in the treatment of post viral syndrome which composition comprises a sterile aqueous solution of highly purified beta-glucuronidase.

2. The use of beta-glucuronidase for the manufacture of a composition for use in the treatment of Acquired Immunodeficiency Syndrome which composition comprises a sterile aqueous solution of highly purified beta-glucuronidase.

3. The use of beta-glucuronidase according to either claim 1 or claim 2, wherein the concentration of beta-glucuronidase in the sterile aqueous solution is in the range of from 50 to 1000 Fishman units per dose delivered to a patient.

4. The use of beta-glucuronidase according to any one of claims 1 to 3, wherein the composition additionally comprises a compound containing at least two hydroxyl groups for controlling the immunological activity of the beta-glucuronidase.

5. The use of beta-glucuronidase according to claim 4, wherein the compound containing at least two hydroxyl groups is 1,3-cyclohexane diol at a concentration in the range of from 3×10^{-10} to 1×10^{-11}g per dose delivered to a patient.

6. The use of beta-glucuronidase according to any one of claims 1 to 5, wherein the composition additionally comprises protamine at a concentration in the range of from 1×10^{-8} to 1000×10^{-8}g per dose delivered to a patient.

7. The use of a composition comprising beta-glucuronidase, 1,3-cyclohexane diol and protamine for the manufacture of a medicament for the treatment of viral carrier state in humans, wherein the medicament

comprises a sterile aqueous phosphate-free buffer solution of from 50 to 1000 Fishman units of beta-glucuronidase, from 1×10^{-8} to 1×10^{-7}g of 1,3-cyclohexane diol, from 1×10^{-8} to 1×10^{-5}g of protamine, optionally, all concentrations being per dose delivered to the patient.

8. The use of a composition comprising beta-glucuronidase, 1,3-cyclohexane diol and protamine for the manufacture of a medicament for increasing the cellular immune response in humans, wherein the medicament comprises a sterile aqueous phosphate-free buffer solution of from 50 to 1000 Fishman units of beta-glucuronidase, from 1×10^{-8} to 1×10^{-7}g of 1,3-cyclohexane diol, from 1×10^{-8} to 1×10^{-5}g of protamine and a suitable dose of antigen, all concentrations being per dose delivered to the patient.

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